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TITLE: Use of Exogenous Progestins and Risk of In Situ and Invasive Breast Cancer

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INTRODUCTION:

Given the large number of women exposed to progestins through either contraceptives or menopausal hormone therapies, clarifying the etiologic role of progestin in relation to breast cancer is of public health importance. This study's two projects will further our understanding of the potential risk of breast cancer associated with progestin use. Project 1 involves the enrollment of 225 *in situ* breast cancer cases 20-44 years of age. Project 2 is a case-control study of women 55-74 years of age that will enroll 435 controls and 870 breast cancer cases, of three different histologic types of breast cancer. We changed our original targeted accrual goal from 1300 to 1305. Originally we said we would recruit 900 cases, with 325 cases in each of three breast cancer case groups (ductal, lobular, and mixed) and 325 controls, for a total of 1300 participants. However, when we reviewed our case accrual we found that the rates of lobular and mixed breast cancer have been falling, so we have adjusted our recruitment targets based on our projected number of lobular and mixed cases. We now project that we will enroll a total of about 435 lobular and mixed cases, so we adjusted our targets for ductal cases and controls. For Project 2, we now plan to enroll 870 cases, with 435 lobular and mixed ductal-lobular cases, 435 ductal cases, and 435 controls, for a total of 1305 participants.

Both projects involve a detailed in-person interview and review and testing of tumor samples for various tumor markers. Project 1 will evaluate the hypothesis that DMPA use, particularly current use and use prior to a first full-term pregnancy, is associated with an increased risk of *in situ* breast cancer. Given that lobular and ductal-lobular carcinomas are more hormonally responsive than ductal tumors, Project 2 will test the hypothesis that hormonally-related risk factors for breast cancer will be more strongly associated with tumors with a lobular component than they will be with pure ductal carcinomas.

BODY:

Task 1. Develop Interview Instrument, Other Study Materials, and Tracking System, Months 1-3:

- a. I will lead the development of the structured interview instruments for both projects. In the development of the questionnaire for Project 1, I will utilize a variety of available historical forms that have been extensively field tested and perfected. The questionnaire for Project 2 will be largely based on the questionnaire we used in our completed study of lobular cancer that this project will build on. Both questionnaires will ascertain information on exposures that occurred prior to each subject's "Reference Date" (the date of diagnosis for cases and a comparable assigned date for controls) including: use of hormones, reproductive history, family history of cancer, medical history, anthropometric characteristics, and demographic information.

Status: Each project's questionnaire has been approved by the DOD and is currently in use in the field.

- b. Several other materials will be prepared for these projects including: approach letters to potentially eligible cases and controls; an interview consent form; a HIPAA compliant authorization to access personal health information; tumor tissue (cases only), medical records, and pharmacy records release forms; and a blood specimen donation consent form.

Status: All the documents listed above were approved for both projects.

- c. Study personnel, including the study manager and interviewers, will be trained on the specific procedures to be used in these projects.

Status: The study manager for each project has been trained and worked closely with the PI to develop all the study materials and assisted in the preparation of submissions for DOD human subjects review.

Interviewers for each project have completed their training and are currently working in the field. The interviewers are well versed on our study specific procedures. They received extensive training on confidentiality, obtaining informed consent, administering the questionnaire, and collecting blood and oral specimens.

- d. Institutional Review Board approval, both locally and from the DOD, will be sought for both studies' protocol and documents.

Status: Local and DOD IRB approval has been obtained for both projects. However, it took much longer to get DOD IRB approval for Project 2 than anticipated (over one year), so the start date and time line for the data collection on Project 2 was significantly impacted. Due to the delay, and our concerns about being able to complete Project 2 within the original time frame, we applied for and DOD funded us for one additional year to complete this study as originally proposed.

- e. We will modify the computerized tracking systems we currently use in our other studies to fit the specific needs of these projects. These systems will allow for up-to-date tracking of study progress and retrieval of information on any aspect of the study as needed. For Project 2 these systems are largely already in place given that it will build on a recently completed study.

Status: The tracking systems are in place and in use for both projects.

Task 2. Finalize Subject Eligibility Criteria, Months 1-3

- a. The population base for both of these studies will be the three-county Seattle/Tacoma metropolitan area in Washington State. All subjects must have been residents of one of these three counties at their reference date.

Status: Complete.

- b. For Project 1, women must be premenopausal and 20-44 years of age at their reference date. For Project 2, women must be postmenopausal and 55-74 years of age at their reference date.

Status: Complete.

- c. Women with a prior history of invasive or *in situ* breast cancer will be excluded.

Status: Complete.

Task 3. Case Identification, Months 3-50

- a. For Project 1, cases are premenopausal women 20-44 years of age diagnosed with *in situ* breast cancer from June 1, 2004 through December 31, 2008. For Project 2, cases are postmenopausal women 55-74 years of age diagnosed with invasive lobular, ductal-lobular, or ductal breast carcinoma from April 1, 2004 to December 31, 2008.

Status: Case identification is underway for each project. For Project 1, we have reduced the range of eligible diagnosis dates by four months because more *in situ* cases have been diagnosed than we originally projected; we will recruit women diagnosed from June 1, 2004 through August 31, 2008. With the number of eligible cases and current participation rates, we assume that the target of 225 interviewed cases will be reached with the revised diagnosis dates.

- b. All cases will be ascertained through the Cancer Surveillance System (CSS), a population-based cancer registry covering 13 counties in western Washington State. CSS is a participant in the NCI SEER Program.

Status: Cases for both projects are being ascertained through the Cancer Surveillance System.

- c. For Project 1, based on recent data, a minimum of 63 eligible *in situ* cases per year are expected during each of the proposed 4.5 years of case ascertainment for an estimated total of 280 eligible cases. For Project 2 accrual, we found that the rates of lobular and mixed breast cancer have been falling, so we have adjusted our recruitment targets based on our projected number of lobular and mixed cases. We now project that we will enroll a total of about 435 lobular and mixed cases, so we adjusted our targets for ductal cases and controls. For Project 2, we now plan to enroll 870 cases, with 435 lobular and mixed ductal-lobular cases, 435 ductal cases, and 435 controls, for a total of 1305 participants. Since ductal carcinoma is more common than both lobular and ductal-lobular carcinoma, an age-matched random sample of ductal cases will be selected for enrollment in this study.

Status: Ascertainment of *in situ* cases for Project 1 will continue with August 2008 being the last eligible diagnosis date. We are able to limit the range of diagnosis dates because the number of eligible cases has been slightly larger than expected. In the complete

ascertainment years of 2005, 2006, and 2007, the number of eligible cases was 74, 68, and 71 respectively. Ascertainment of lobular, ductal, and mixed lobular-ductal cases for Project 2 is progressing as expected, but our enrollment numbers are not where we originally projected them to be at this point given the unanticipated delay in obtaining DOD IRB approval for this study.

- d. From our prior experience with breast cancer studies, we anticipate that at least 80-85% will agree to be interviewed. Hence, a minimum of 225 of the eligible 280 cases eligible for Project 1, and 435 of the 540 eligible cases in each of the histology groups for Project 2 would agree to participate.

Status: Case participation in both projects is going well and at the expected rates.

- e. Twice each month CSS files identifying newly diagnosed potentially eligible cases of breast cancer will be downloaded to our personal computers and reviewed by the study manager.

Status: CSS files for each project are downloaded and reviewed by the study managers every month.

- f. The physicians of all eligible cases will receive a letter requesting permission to interview their patient.

Status: Each project is sending physician letters using approaches approved by both our local IRB and the DOD IRB.

- g. The cooperation of area physicians is crucial to the success of studies such as the one proposed. The physician response rates achieved by this research unit have consistently been high (greater than 99%).

Status: Physician cooperation for both projects is going well and at the expected rates.

Task 4. Control Identification, Months 3-50

- a. General population controls with no prior history of breast cancer who are 20-44 years of age (Project 1) and 55-74 years of age (Project 2) will be identified through random digit dialing (RDD). However, all of the work related to controls for Project 1, including identification, approach, and data collection will be funded by the parent R01 (NCI) grant, and not by the Era of Hope Scholar Award.

Status: RDD controls are currently being identified for each project.

- b. We will use a system that automates the administration, execution, and tracking of the RDD process.

Status: The automated system is in place and operational for both projects.

- c. This control identification procedure has been used successfully for prior and on-going studies in our research group. For example, in a recent breast cancer case-control study, our interview response rate (number interviewed/number eligible) among women 35-44 was 88% for controls and 90% for cases.

Status: Control identification for both projects is going well and at the expected rates.

Task 5. Approach to Study Subjects, Months 4-53

- a. Cases and controls will be approached about each of these studies through a letter describing the study's purpose and procedures, and advising them that an interviewer will call soon.

Status: Cases and controls are currently being approached for each project. (See enrollment table attached.)

- b. Within one week of the initial mailing, a trained interviewer will call the subject to answer any questions, verify eligibility, and schedule the interview. Then a letter confirming the appointment will be sent to subjects.

Status: These processes are underway for each project.

- c. We will attempt to complete all interviews in the woman's home. If this is not possible, the interviewer will arrange to have the interview take place in a location agreeable to the subject.

Status: Most interviews have taken place in the participants' homes. A few have been conducted at FHCRC, at work, or another location chosen by the participant. Two interviews for Project 1 were completed by telephone because the case had moved out of the study area. Seventeen of the interviews for Project 2 were completed by telephone because the case had moved out of the study area or because the respondent refused to allow the interviewer in her home.

- d. In our past studies we have been able to interview 60% of subjects willing to participate within three months of initial contact, and 93% within six months.

Status: Enrollment is progressing as expected for each project.

Task 6. Conduct of Interviews, Months 4-55

- a. For Project 1, 225 *in situ* cases will be interviewed. For Project 2, 870 cases, including 435 lobular and mixed ductal-lobular cases and 435 ductal cases, will be interviewed. These sample sizes were selected to provide both studies with adequate statistical power to evaluate each of their specific aims.

Status: Case interviews are underway for each project. Please see the attached enrollment table. Again, enrollment is behind schedule for Project 2 due to the delay in obtaining DOD IRB approval for this study, but we are confident we can complete the study with the additional year of funding that was provided to us by DOD.

- b. At the time of the interview a consent form will first be reviewed and then signed by both the study subject and the interviewer.

Status: Every completed interview has a consent form signed by the participant and the interviewer.

- c. The interview will then be conducted.

Status: Interviews are being conducted for each project. (See the enrollment table for each project attached.)

- d. HIPAA compliant authorizations to collect personal health information will be sought including:
 - i. A tumor tissue release so that specimens can be ascertained and tested for a variety of molecular markers in the Porter lab;
 - ii. A medical records release so we can review medical records from locations where subjects have been prescribed contraceptives;
 - iii. A pharmacy records release that gives us permission to contact their usual pharmacies to verify and supplement reported medication use.

Status: HIPAA authorizations, as well as tumor tissue, medical records, and pharmacy records releases, have been signed by the majority of participants. (See the enrollment table attached.)

- e. All subjects will be asked to donate three tubes of blood. Subjects who refuse will be asked if they are willing to provide us with an oral tissue sample. All blood and oral specimens will be transported to the Fred Hutchinson Cancer Research Center (FHCRC) Specimen Processing Laboratory within 24 hours of collection for processing and storage.

Status: Most of the participants interviewed for each project have agreed to donate a blood sample. Most participants that refused to donate blood agreed to donate an oral specimen. (See the enrollment table attached.)

- f. Interviewers will edit each interview within 3 days of their completion. Next, one of our staff members who has extensive editing experience will edit and code the completed questionnaires. Lastly, the study manager will conduct a final edit of all questionnaires and determine which subjects need to be recontacted so that missing or incomplete data can be collected.

Status: The interviewers, editors, and study manager for each project are conducting edits and final review for all completed interviews.

- g. Telephone validation interviews on a randomly selected 10% of interviewed women will be conducted within one month of the date that original interviews were completed to determine if the answers to selected questions are comparable to those given during the interview.

Status: 10% of the completed interviews for each project have been validated over the phone.

Task 7. Tissue Collection, Pathology Review and Laboratory Testing of Tumor Specimens, Months 4-55

- a. We will request that histology slides, tissue blocks, and pathology reports from the breast cancer cases we enroll be sent to the Porter lab at FHCRC from regional pathology laboratories.

Status: Tissue collection for each project is in process.

- b. Based on our previous studies, we anticipate that at least 95% of subjects will consent to release their tissue. We have considerable experience in the acquisition of tumor blocks, and we anticipate that we will be able to collect tumor tissue for at least 75% of all cases.

Status: Most of the participants interviewed for each project have signed a tumor tissue release. (See the enrollment table attached.)

- c. Whenever possible a single representative block is selected for all laboratory studies. In summary, we will:
 - i. Conduct pathology reviews on all tumors;
 - ii. Assay expression of ER α , ER β , PR, and e-cadherin using immunohistochemistry (IHC) on conventional slides;
 - iii. Harvest tissue cores from selected tissue blocks for construction of tissue microarrays (TMA). Biomarkers most relevant to the relationship between DMPA and breast cancer risk at the time TMA construction is complete will be evaluated on these slides. These TMA slides will also provide a valuable resource for future studies since they allow for high throughput testing of additional markers and offer maximum flexibility for studying new markers as they emerge and develop.

Status: For Project 1, we have collected tumor blocks for 76 women, and continue with ongoing tissue requests. For Project 2, we have collected 402 tissue blocks and continue with ongoing tissue requests. Tissue testing has begun and the lab continues to log in samples in preparation for testing. It is most efficient to do this testing in large batches. Given the delay in participant enrollment resulting from the delay in obtaining DOD IRB approval for Project 2, the laboratory portion of this project has been delayed, but will be completed as originally proposed with the additional year of funding we received from DOD.

Task 8. Data Analysis and Manuscript Preparation, Months 51-60

- a. For Project 1, unconditional logistic regression will be used to compute odds ratios (and 95% confidence intervals) that characterize the relationship between DMPA use and *in situ* breast cancer risk. For Project 2, polytomous logistic regression will be used to compute odds ratios (and 95% confidence intervals) that characterize the relationship between hormone therapy use and risk of the three histologic types of breast cancer of interest in the same statistical model.

Status: Not scheduled to begin until month 51, but for Project 2, the start date will be delayed because of the additional year granted to finish the interviewing.

- b. Systematic assessment of the effect of potential confounders (including body mass index, body weight, parity, reproductive factors, and oral contraceptive use) on the estimates obtained will be performed.

Status: Not scheduled to begin until month 51, but for Project 2, the start date will be delayed because of the additional year granted to finish the interviewing.

- c. Two approaches will be used to assess effect modification. First, we will simply stratify our logistic regression analyses by the effect modifier of interest so that risks specific to each stratum of the potential effect modifier can be calculated. Second, we will include an interaction term between the exposure of interest and the effect modifier so that the statistical significance of the observed interaction (effect modification) can be determined.

Status: Not scheduled to begin until month 51, but for Project 2, the start date will be delayed because of the additional year granted to finish the interviewing.

- d. I will lead the preparation of the multiple anticipated manuscripts that will describe the results of these two projects.

Status: Not scheduled to begin until month 51, but for Project 2, the start date will be delayed because of the additional year granted to finish the interviewing.

KEY RESEARCH ACCOMPLISHMENTS:

Data collection is underway for each project.

REPORTABLE OUTCOMES:

None.

CONCLUSION:

None.

REFERENCES:

None.

APPENDICES:

None.

SUPPORTING DATA:

None.

Table 1
Use of Exogenous Progestins and Risk of *In Situ* and Invasive Breast Cancer
September 15, 2009

	Project 1		Project 2			
	Cases	Percent	Cases	Percent	Controls	Percent
Total Ascertainment from CSS or RDD	330		1431		675	
Ineligible	29	8.8%	211	14.7%	101	15.0%
Eligible	301	91.2%	1220	85.3%	574	85.0%
 Non Participants (percent of Eligibles)	 55	 18.3%	 204	 16.7%	 153	 26.7%
Deceased prior to contact	1		49		1	
RDD refusal	0		0		75	
Physician refusal	1		4		0	
Unable to participate or subject-level refu	53		151		77	
 In Process (percent of Eligibles)	 61	 20.3%	 250	 20.5%	 65	 11.3%
Review of CSS files	7		7		0	
Physician letter to be mailed	6		0		0	
Subject letter to be mailed	33		134		8	
Assigned to interviewer	15		109		57	
 Enrolled (percent of Eligibles)	 185	 61.4%	 766	 62.8%	 356	 62.0%
Interview completed	185		766		356	
Biological sample obtained*						
Blood sample	144		645		296	
Oral sample	48		76		38	
Tissue sample	76		402		not applicable	
HIPAA Authorization signed	182		759		344	
Tumor Tissue Release signed	179		757		not applicable	
Medical Records Release signed	179		744		329	
Pharmacy Records Release signed	148		736		326	

*This reflects complete samples obtained. Additional samples are currently in process.

Certificate of Environmental Compliance (CEC)

This Certificate of Environmental Compliance shall be executed by the institution's official responsible for environmental compliance. The Council on Environmental Quality (CEQ) regulations (40 CFR 1500-1508) that implement the National Environmental Policy Act (PL 91-190, as amended) require all federal agencies to examine possible environmental consequences of their proposed and ongoing actions.

- 1 One CEC (this form) must be submitted for each site conducting research under the submitted research proposal. This includes all subcontractors.
- 2 If you have any questions concerning the generation or applicability of a CEC, please e-mail or call (301-619-2004) the USAMRMC Command Environmental Coordinator.

The offeror currently **X IS** ☐ **IS NOT** in compliance with applicable national, state, and local environmental laws and regulations. (If not in compliance, attach details and evidence of approved mitigation measures.) The offeror has examined the activities encompassed within the proposed action for compliance with environmental laws and regulations.

PROPOSAL TITLE: Use of Exogenous Progestins & Risk of in situ & Invasive Breast Cancer: Project 1 & 2

PRINCIPAL INVESTIGATOR: Christopher Li

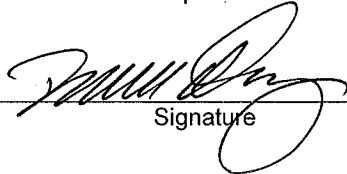
The offeror states that the conduct of the proposed action:

- 1 WILL NOT violate any applicable national, state, or local environmental law or regulation, and
- 2 WILL NOT have a significant impact on the environment.

The offeror agrees that if the work required under the proposed action at any time results in a significant impact on the environment or a violation of any applicable environmental law or regulation, the offeror will immediately take appropriate action, to include notifying and/or coordinating with the appropriate regulatory agencies as required by law and notifying the Grants Officer.

Donald Wang, Director, Environmental Health & Safety

Name and Title of Official Responsible for Environmental Compliance


Signature

Sept. 22, 2009
Date

Name of Organization: Fred Hutchinson Cancer Research Center